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CAMBRIDGE UNIV (ENGLAND) DEPT OF ZOOLOGY J E TREHERNE
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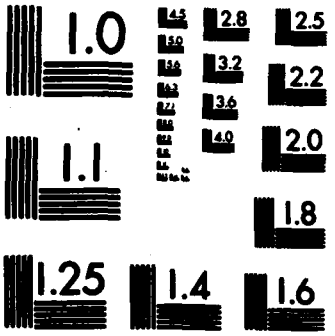

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

CELLULAR MECHANISMS OF CENTRAL NERVOUS MODULATION

by Dr. J.E. Treherne

Department of Zoology, University of Cambridge, U.K.

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Scientific activities

We have recently been studying the mechanisms of cellular repair and regeneration in the nervous system using the relatively simple cockroach nerve cord as a model system. Our primary aim has been to elucidate the mechanisms of glial repair. This involves an understanding of glial-neurone interactions and, for this reason, we have studied neuronal regeneration following surgical lesioning.

Unlike mammalian central neurones, which show only limited regenerative growth, those of the insect central nervous system can emit sprouts which extend for relatively large distances. The giant interneurones of the cockroach are particularly well suited for such studies.

It has been suggested that the entry of calcium ions plays an important regulatory role in neurite extension. Meiri, Spira & Parnas (Science 122, 709-712, 1981) have claimed that their observations on electrophysiological responses of regenerating cockroach axons are consistent with this hypothesis They reported the transient appearance of calcium-dependent action potentials (between 7 and 60 hours after axotomy) near the regenerating types of severed giant axons.

The appearance of Ca-channels near the tips of severed axons in the early stages of regeneration raises a number of interesting questions. What are the characteristics of these channels? Is their appearance a purely local response, perhaps involving modification of existing ion-channels, or are these Ca-channels transported from the cell body? If it is not a local response, then what is the nature of the signal, or signals, that initiate the mobilization, transport and incorporation of the channels at the axonal tips?

To try and answer some of these questions we have repeated and extended the investigation of Meiri et al. (1981) and examined the ion

specificity of excitability at different stages of regeneration near the tips of proximal and distal segments of ligatured and unligatured cut axons.

In ligatured connectives, severed in vitro, on the distal side from the cell bodies, giant interneurones maintained normal resting and action potentials for at least 5h. In connectives ligatured and severed in vivo, Na-dependent, overshooting action potentials could be recorded which, after 24h, were blocked by TTX (3×10^{-7}M) but not by cobalt (20 mM). Similar results were obtained after 48 and 72h. Axotomized axons in ligatured connectives (cut on the side of the ligature proximal to the cell body) maintained Na-dependent action potentials for up to 20 days.

In some unligatured connectives (cut close to the site of intracellular recording proximal to the cell bodies) axonal resting and action potentials were maintained for at least 5h - suggesting that the cut ends of the axons had sealed. In other preparations, however, the resting potential of the cut axons declined rapidly, accompanied by a loss of the action potentials. It was impossible to restore the action potential by hyperpolarising the membrane to -80 mV after less than 48h. After this time (51h) action potentials could be restored, which could be blocked by TTX, but not by cobalt.

The above observations do not support the hypothesis of Meiri et al. (1981) for the appearance of transient, calcium-dependent action potentials near the tips of regenerating giant interneurones. Similar results have also been recently obtained from another laboratory (M.E. Spira, personal communication).

The effect of cutting giant interneurones at different distances from their cell bodies was also investigated. There was no apparent relation between the responses of the giant interneurones and the site of lesion. Sprouting of the giant interneurones (visualized in cobolt filled preparations, 14, 21 and 28 days after cutting) was observed both at the tip of the axon and also at sites some distance from the lesion. No

changes in the cell body, or its dendritic tree, in the terminal ganglion were seen, but sprouting was observed in other ganglia.

The above results indicate that axonal sprouting can occur in the cockroach C.N.S. without a measurable, transitory, incorporation of voltage-sensitive calcium channels. This implies that neurite elongation can occur in the absence of calcium entry through specific voltage-sensitive channels.

Publications

Leech, C.A. & Treherne, J.E. (1984). Growth and ion-specificity of excitability in regenerating cockroach giant axons. J. exp. Biol. (in press).

Smith, P.J.S. & Howes, E.A. (1984). Glial to axon protein transfer in an insect. (submitted to J. Cell Sci.).

Treherne, J.E., Harrison, J.B., Treherne, J.M. & Lane, N.J. (1984). Glial repair in the central nervous system of an insect: effects of surgical lesioning. (submitted to J. Neuroscience).

Smith, P.J.S., Leech, C.A. & Treherne, J.E. (1984). Glial repair in an insect central nervous system: effects of selective glial disruption. (submitted to J. Neuroscience).

Future Research Plans

The final stages of experiments on the effects of octopamine on the electrophysiological responses of the perineurial glia are in progress. Electrophysiological, ultrastructural and autoradiographic investigation will be continued on the processes of glial repair following selective disruption with a glial toxin.

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